THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
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DEPARTMENT OF BIOCHEMISTRY

March 4, 1958

Dr. Joshua Lederberg
Department of Medical Genetics
Genetics Building
University of Wisconsin
Madison 6, Wisconsin

Dear Joshua:

The problem of growing competent cells in media other than Levinthal broth has so far been very disappointing. After some intensive original experiments we used a modified medium which I described in a previous letter, and except for an occassional foray have continued to use this medium for preparing competent cells.

The procedure we use is as follows:

(1) Inoculate approximately 0.5 ml. of fully grown culture into 50 ml. of Levinthal broth (1 part Levinthal stock and 1 part Eugonbroth).

(2) Grow cells with aeration to a concentration of 10^9 cells per ml. On our Coleman Junior at 650 mu with an adapter for 10 x 100 mm. pyrex tubes this corresponds to a turbidity reading of .09 (O.D).

(3) The cell suspension is then transferred to a large test tube or small flask which is kept at 36-37°C. for an additional 90 minutes. Some growth does occur usually to about 1.5×10^9/ml. Cells grown aerobically would grow to about 6×10^9/ml. in this period of time.

The method of preparing competent cells a la Alexander and Leidy consists of growing the cells until they ~~almost~~ approach saturation. We reasoned that the competency might be more related to aeration or growth limitation than to cell number; consequently, we restricted the aeration of the culture during early log phase growth and found the cells to be competent. Actually the culture may be grown to any log phase concentration, placed under non-aerobic conditions, and the cells will become competent.

With regard to the stability of DNA, we have kept preparations in the refrigerator for almost three years (in fact, the first preparation I ever made) and the potency of this DNA is as good as ever.

It does not matter when the DNA is added to the cells as long as they are competent for some period during exposure. It is important, however, that there be enough free divalent cations to give about .002 M excess over any chelating agents such as citrate which might be used to stabilize the DNA. We generally use Mg++ for this purpose; Ca++ is also O.K. Ca++ would be better in the case of pneumococcus transformations since there appears to some DNAse liberated by Pn.

I am forwarding some Levinthal stock which should be diluted 1:1 with Eugonbroth, and a sample of DNA. If for any reason you have difficulty in preparing competent cells, I can ship you some of ours packed in dry ice. In this case it will be necessary to coordinate air freight schedules so that someone can pick up the package as soon as it arrives.

If it is convenient, would you please try to reserve space in the dormitory for me for the period of the symposium. If you are crowded for dormitory space, it would suit me quite well to have whatever hotel accommodations are available. I am planning to give a course in Genetics next fall to people in the Medical Institutions, and I would appreciate the opportunity to obtain a closer look at some of the areas under discussion.

If you have any difficulties whatsoever, please do not hesitate to write.

Sincerely,

Sol H. Goodgal

SHG:csb